long-lasting competence factor Dlx3b–Dlx4b and adopt and maintain an ear fate.

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Math5 expression and function in the central auditory system

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Math5 is a basic helix–loop–helix (bHLH) transcription factor necessary for retinal ganglion cell (RGC) and optic nerve development. Using *Math5-lacZ* knockout mice, we have identified additional expression domains for *Math5* in functionally connected structures of the central auditory system. The cytoplasmic *Math5-lacZ* reporter is expressed in the cochlear nucleus (CN) during embryonic development, and in the CN, medial nucleus of the trapezoid body (MNTB), lateral superior olive (LSO), and lateral lemniscus (LL) in the adult hindbrain. *Math5-lacZ* is also expressed in the developing cochlea from E14.5 to E16.5 and in a small subset of auditory nerve fibers in adult mice. The hindbrains of *Math5* mutants appear grossly normal with the exception of the CN. Overall CN dimensions are unchanged, but *lacZ* positive cells are significantly smaller in *Math5*−/− mice as compared to *Math5*+/− mice. This change in cell size may reflect abnormal function. Based on marker coexpression as well as anatomical position within the CN, we propose that these *Math5-lacZ* cells represent a subset of spherical and globular bushy cells. The projection pattern of these cell types is consistent with the observed *Math5-lacZ* hindbrain expression. We evaluated the auditory brainstem response (ABR) in *Math5* mutants in a BALB/cJ congenic background. Hearing thresholds of *Math5*−/− mice were similar to those of wild type and heterozygous mice, but the interpeak latencies for peaks II–IV were significantly altered. These temporal changes are consistent with a higher level auditory processing disorder, potentially involving the integration of binaural sensory information.

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Involvement of the forkhead transcription factor FoxN4 in *Xenopus* retinal progenitor cell development

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The forkhead transcription factor FoxN4 is primarily expressed in the developing retinal progenitor cells of *Xenopus laevis* embryos, from specification until maturation. The purpose of our studies is to investigate the role of FoxN4 in *Xenopus* retinal development. Morpholino oligonucleotides and synthetic RNAs were injected into 4-celled embryos. Embryos were cultured to desired stages, fixed in paraformaldehyde, and analyzed. Gene expression was visualized by in situ hybridization using antisense riboprobes and whole fixed embryos or 8 μM sections of paraffin-embedded embryos. Immunocytochemistry was performed using 8 μM sections of paraffin-embedded embryos and visualized by immunoperoxidase reactions. Histology was visualized by hematoxylin and eosin staining of 8 μM sections of paraffin-embedded embryos. Embryos injected with FoxN4 antisense morpholino oligonucleotide exhibited a reduction in eye size and loss of marginal zone progenitor cells. Embryos overexpressing FoxN4 exhibited a variety of phenotypes including reduction in eye size and/or extra or ectopic retinal tissue and neural tubes. Smaller eyes were primarily observed in embryos injected with higher doses of FoxN4 RNA at early stages. Extra retinal tissue was observed within the optic nerve, optic cup and neural tube. In some cases, FoxN4-injected embryos developed whole ectopic eyes. Extra neural tubes were accompanied by ectopic neural progenitor cells. These data suggest that FoxN4 plays a role in the development of neural and retinal progenitor cells in *Xenopus*.

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Exploration of Math5 regulation and the developing visual system using a GFP-expressing transgene

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Neural development in the mouse retina is dependent on the transcriptional regulation of neural progenitors. Math5, a homologue of *Drosophila* atonal, is a bHLH transcription factor expressed in retinal ganglion cells (RGCs) and in the cochlear nucleus of the hindbrain. RGCs, which convey information from the retina to the brain via the optic nerve, require the expression of Math5 for proper development. Regulatory regions of the Math5 gene were used to drive GFP in transgenic mice. Due to the purdurance and cytoplasmic diffusion of GFP, we observe live GFP fluorescence within RGC axons in the optic nerve, chiasm, and tract. We explored the path of these axons using live fluorescence and immunostaining and document expression in the superior colliculus and developing lateral geniculate nucleus. Interestingly, one transgenic construct lacking 3′ putative regulatory elements also expresses GFP in regions of the nervous system not associated with Math5 expression. We compared these expression domains to those of Math1, another atonal homologue, and to Pax6, an upstream regulator of Math5 expression, by immunostaining. Math5-GFP and Math1 are coexpressed in certain neurons of the rhombic lip, whisker barrels, and in inner ear hair cells. Math5-GFP and Pax6 are coexpressed in particular neurons of the neocortex, thalamus, ventral spinal